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EXAMINER	
STRZELECKA, TERESA E	
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1637	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/544,161	BERLIN, KURT	
	Examiner	Art Unit	
	TERESA E. STRZELECKA	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 May 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,9-29,31-36 and 40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,9-29,31-36 and 40* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/3/09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This case has been transferred to examiner Teresa Strzelecka, since examiner Baughman left the USPTO.

2. This office action is in response to an amendment filed May 27, 2009. Claims 1-40 were previously pending. Applicants amended claims 1, 9-29, 31-36 and 40 and canceled claims 2-8, 30 and 37-39. Claims 1, 9-29, 31-36 and 40 are pending and will be examined. Applicants' amendments overcame all of the previously presented rejections.

3. This office presents new grounds for rejection necessitated by amendment.

Information Disclosure Statement

4. The information disclosure statement (IDS) submitted on June 3, 2009 was filed after the mailing date of the non-final office action on November 21, 2008. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claim 40 is rejected under 35 U.S.C. 102(b) as being anticipated by Herman et al (US 6,265,171; cited in the previous office action).

Regarding claim 40, Herman et al. teach a kit consisting of a reagent containing a bisulfite, and primers for the amplification, as well as nucleotides and other components necessary to perform the amplification reactions (see col. 21, lines 45-67 through col.22, lines 1-9). Since the limitation of the initial concentration of the dCTP or dGTP is an intended use limitation, referring to the reaction conditions, the claim is anticipated by Herman et al.

6. Claim 40 is rejected under 35 U.S.C. 102(b) as being anticipated by Das et al. (US 6,143,504; cited in the previous office action).

Das et al. teach a kit comprising bisulfite, primers and a nucleotide mixture (see col.4, lines 24-34). Since the limitation of the initial concentration of the dCTP or dGTP is an intended use limitation, referring to the reaction conditions, the claim is anticipated by Das et al.

7. Claim 40 is rejected under 35 U.S.C. 102(b) as being anticipated by Cottrell (US 6,960,436; cited in the previous office action).

Cottrell teaches a kit comprising bisulfite, primers and a nucleotide mixture (see col.7, lines 1-5; and col.10, lines 28-55). Since the limitation of the initial concentration of the dCTP or dGTP is an intended use limitation, referring to the reaction conditions, the claim is anticipated by Cottrell.

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1. Claims 1, 10-12, 14, 19-22, 26-27, 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action).

A) Regarding claims 1, 20, and 26-27, Herman et al. teach a method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:

a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged (see abstract and col.3);

the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA (see abstract and col.3, and col.5-6, where it explains that amplification distinguishes between modified and unmodified DNA); and

the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity (col.6, lines 1-6, Fig.2A-E; col.4, lines 29-48; col.9, lines 51-67).

Regarding claim 10, Herman teaches the method further characterized in that the denaturing temperature lies below 90 °C in the PCR amplification (see col.8, lines 17-18).

Regarding claim 11, Herman teaches the method further characterized in that the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of an individual (see col.7, lines 59-62).

Regarding claims 12 and 14, Herman teaches the method further characterized in that the chemical treatment is conducted with a bisulfite, disulfite, or hydrogen sulfite containing solution, and that in the chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present (see col.6, lines 7-25).

Regarding claim 19, Herman teaches the method further characterized in that the polymerase used has no 5'-3' exonuclease activity (see col.9, lines 5-6, where T4 polymerase does not have 5' to 3' exonuclease activity).

Regarding claims 21-22, Herman teaches the method further characterized in that the primers in the amplification distinguish between target DNA and background DNA, and that the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment (see abstract and col.6, lines 26-37).

Regarding claims 31-32, Herman teaches the method further characterized in that the amplificates themselves bear a detectable label for the detection, and wherein such labels are fluorescent labels (see col.14, lines 6-11, wherein the primers have the labels and therefor the amplificates formed from a reaction using such primers comprise the labels; and col.22, lines 57-59, same except the detectable labels are biotin).

B) Herman et al. do not teach reaction mixtures where the concentration of either dCTP or dGTP is at most half the concentration of the average initial concentration of the other nucleotides.

C) Radlinska et al. teach detection of 5-methylcytosines by bisulfite conversion and subsequent amplification using reaction mixtures entirely lacking dGTP (Abstract; page 325, paragraphs 3-5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the method of Radlinska et al. to detect the unmethylated cytosines in the method of Herman et al. The motivation to do so is provided by Radlinska et al. (page 329, second paragraph):

"The main difference between our new method and the original genomic sequencing protocol relies on direct localization of 5-MeC in the primary product of the bisulfite treatment instead of the secondary product (of PCR amplification). The primer extension mix contains only three deoxynucleotides (dATP, dCI'P, dTTP) and lacks dGTP. Elongation of the synthesis product is terminated selectively when 5-MeCs occur in template DNA, since those are the only positions where cytosines were not converted to uracil by bisulfite treatment of the template strand and dGTP is needed to continue the primer extension (Fig. 1). Therefore distinct bands appearing in the ran-off lane in positions corresponding to the nucleotide complementary to the base following 5-MeC on the template strand allow direct localization of the methylated residues (Fig. 2)."

9. Claims 1, 11-12, 14, 20-29, 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Eads et al. (Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action).

A) Regarding claims 1, 20, and 26-27, Eads et al. teach a method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:

a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged (see pg.ii, "Sodium bisulfite conversion and COBRA analysis");

the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA (see pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences," where the primers used target either methylated or unmethylated CpG dinucleotide sites); and

the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity (see abstract, and pg.ii-iii, "Quantitative RT-PCR and microsatellite instability analysis and Figure 3).

Regarding claim 11, Eads teaches the method further characterized in that the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of an individual (see abstract and pg.ii, "Sample Collection").

Regarding claims 12 and 14, Eads teaches the method further characterized in that the chemical treatment is conducted with a bisulfite, disulfite, or hydrogen sulfite containing solution, and that in the chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present (see pg.ii, "Sodium bisulfite conversion and COBRA analysis").

Regarding claims 21-22, Eads teaches the method further characterized in that the primers in the amplification distinguish between target DNA and background DNA, and that the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment (see pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences," where the primers used target either methylated or unmethylated CpG dinucleotide sites; and Figure 3).

Regarding claim 23-27, Eads teaches the method further characterized in that additionally at least one reporter oligonucleotide is used in the amplification whose fluorescence properties change as a consequence of the amplification [claim 23]; wherein a Taqman assay or a LightCycler assay or an assay with the use of Molecular Beacons is conducted to conclude upon the methylation state at the last step of the method [claim 24]; that the reporter oligonucleotide bears at least one fluorescent label [claim 25] (see abstract, and pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences").

Regarding claims 28 and 29, although Eads does not specifically discuss the method wherein the background DNA is present in 100X or 1000X concentration in comparison to target DNA, he does discuss conducting a similar assay where fully methylated human sperm DNA (by treatment) is serially diluted in 10-fold increments up to 1:100,000 with untreated, unmethylated human sperm DNA in order to test the sensitivity and quantitative accuracy of the MethyLight technique (see Figure 4). Eads explains that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples (see

pg.v, "Sensitivity and quantitative accuracy of MethyLight technology"). Therefore, although in the Eads method both the types of DNA are not chemically treated, one of skill in the art could reason that his example achieves the same predictable result of being able to detect methylation patterns in samples which are highly contaminated with non-target DNA up to 1000X.

Regarding claims 31 and 32, Eads et al. teach amplificates carrying fluorescent labels (Fig. 1).

B) Eads et al. do not teach reaction mixtures where the concentration of either dCTP or dGTP is at most half the concentration of the average initial concentration of the other nucleotides.

C) Radlinska et al. teach detection of 5-methylcytosines by bisulfite conversion and subsequent amplification using reaction mixtures entirely lacking dGTP (Abstract; page 325, paragraphs 3-5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the method of Radlinska et al. to detect the unmethylated cytosines in the method of Eads et al. The motivation to do so is provided by Radlinska et al. (page 329, second paragraph):

"The main difference between our new method and the original genomic sequencing protocol relies on direct localization of 5-MeC in the primary product of the bisulfite treatment instead of the secondary product (of PCR amplification). The primer extension mix contains only three deoxynucleotides (dATP, dCTP, dTTP) and lacks dGTP. Elongation of the synthesis product is terminated selectively when 5-MeCs occur in template DNA, since those are the only positions where cytosines were not converted to uracil by bisulfite treatment of the template

strand and dGTP is needed to continue the primer extension (Fig. 1). Therefore distinct bands appearing in the ran-off lane in positions corresponding to the nucleotide complementary to the base following 5-MeC on the template strand allow direct localization of the methylated residues (Fig. 2)."

10. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), or Eads et al. (Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), as applied to claim1 above, and further in view of Yuanxiang et al. (Biotechniques, May 1997, Vol.22, pp.850-853; cited in the previous office action).

A) The teachings of Herman et al., Eads et al. and Radlinska et al. are discussed above. Although these references discuss the amplification reactions comprising sequencing (see col. 22, lines 37-67 of Herman, and pg.iii, "Bisulfite genomic sequencing"), they do not particularly state that such reactions include terminating dideoxynucleotides.

B) Yuanxiang et al. discuss a method of analyzing methylation in DNA by a reaction which distinguishes between cytosine and 5-Methylcytosine in bisulfite-modified DNA (see title). Such a reaction comprises using terminating dideoxynucleotides during the reaction (see Figure 1 and pg.851, right column and pg.853, last paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method of Herman et al. and Radlinska et al. or Eads and Radlinska et al. to include terminating dideoxynucleotides in the amplification because Yuanxiang

demonstrates that it was conventional in the art at the time of the invention to include terminating dideoxynucleotides into amplification reaction for the benefit of sequencing the DNA when analyzing methylation. The skilled artisan would have had a reasonable expectation of success in further including terminating dideoxynucleotides in the amplification of Herman et al. and Radlinska et al. or Eads et al. and Radlinska et al. for the added benefit of sequencing the DNA for determining the methylation state.

11. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action) or Eads et al (Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), as applied to claims 1 and 12 above, and further in view of Guetig (US 2004/0248120; cited in the previous office action).

A) The teachings of Herman et al. and Radlinska et al. and Eads et al. and Radlinska et al. are discussed above. None of the references teach the method where the chemical treatment is conducted after embedding the DNA in agarose.

B) Guetig explains that treating DNA with bisulfite after it is embedded in agarose was a conventional practice in the art at the time of the invention (see paragraph [0017]). Guetig explains that such a method allows for the prevention of the diffusion and renaturation of DNA to occur during the treatment process, thereby allowing the DNA to stay single-stranded, which is required for bisulfite treatment.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the agarose-embedding method of Guetig in the method of Herman et al. and Radlinska et al. and Eads et al. and Radlinska et al., since such treatment prevented renaturation of the double strands, and since the success of full bisulfite conversion depends on the DNA remaining single-stranded, the use of agarose increases probability of full conversion of unmethylated cytosines to uracil.

12. Claims 15-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action) or Eads et al (Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), as applied to claim 1 above, and further in view of in view of Orum et al. (Nuc. Acids Res., 1993, Vol.21, No.23, pp.5332-5336; cited in the previous office action).

A) The teachings of Herman et al., Eads et al. and Radlinska et al. are discussed above. None of the references teach amplification conducted in the presence of at least one other oligonucleotide or a PNA oligomer as recited in claim 15, 16, 17, or 18.

B) Orum et al teach a method of PCR clamping in which PCR amplification is inhibited by the use of primers that cannot be extended. Orum et al. explain how the PNA will compete with the PCR primer for any primer sites in the genome, thereby suppressing any occurence of non-specific background in the PCR process directed by this primer (see pg.5336, second paragraph). Orum et al. also discuss how the method can be applied in reverse manner, where the PNA clamps target non-mutated genes, thereby leading to reduction of unwanted background

amplification (see pg.5336, last paragraph). Orum et al. use the method to detect mutations, the principle of the method uses such PNA oligomers in the detection of particular targeted nucleotide bases in the genome, and one of skill in the art could reasonably conclude that the method could also be used to analyze targeted cytosines, as in the method of Herman et al. and Radlinska et al. or Eads et al. and Radlinska et al.

Therefore, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to modify the method of Herman et al. and Radlinska et al. or Eads et al. and Radlinska et al. to utilize further oligonucleotides or PNA oligomers that impede the binding of primer oligonucleotides to background DNA because Orum et al. demonstrate the benefits of utilizing PNA oligomers in single base detection amplification reactions in order to reduce background amplification and increase signal strength.

13. Claims 28-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), as applied to claim 1 above, and further in view of Eads et al. (Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii; cited in the previous office action)

A) The teachings of both Herman et al. and Radlinska et al. are discussed above. Herman et al. do not discuss the method wherein the background DNA is present in 100X or 1000X concentration in comparison to target DNA.

B) Eads discusses conducting a similar assay where fully methylated human sperm DNA (by treatment) is serially diluted in 10-fold increments up to 1:100,000 with untreated, unmethylated human sperm DNA in order to test the sensitivity and quantitative accuracy of the

MethyLight technique (see Figure 4). Eads explains that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples (see pg.v, "Sensitivity and quantitative accuracy of MethyLight technology"). Therefore, although in Eads method both the types of DNA are not chemically treated, one of skill in the art could reason that his example achieves the same predictable result of being able to detect methylation patterns in samples which are highly contaminated with non-target DNA up to 1000X.

One of skill in the art would have been motivated to modify the method of Herman et al. and Radlinska et al. to conduct the assay where the background DNA is present in 100X or 1000X concentration in comparison to target DNA because Eads et al. demonstrate that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples. Therefore, the skilled artisan would have had a reasonable expectation of success in conducting the method of Herman et al. and Radlinska et al. using background DNA at 100X or 1000X concentrations in comparison to target DNA for the benefit of conducting the assay in a manner that mimicked one using non-microdissected, heterogeneous tissue samples in order to ensure sensitivity and high quantitative accuracy in the method.

14. Claims 33-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Herman et al (US 6,265,171; cited in the previous office action) and Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action), as applied to claims 1

and 31 above, and further in view of Olek et al. (WO 01/77378 A2, published October 18, 2001; cited in the previous office action).

A) The teachings of Herman et al. and Radlinska et al. are discussed above. Although Herman et al. teach a method where the amplificates themselves bear a detectable label for detection, they do not teach the labels being radionuclides, or where they are removable mass labels which are detected in a mass spectrometer. Although they teach primers comprising biotin groups, where the amplificates can then be bound to streptavidin-coated beads following amplification (col.22, last paragraph), they do not teach the reaction occurring on the beads.

B) Olek et al. discuss methods of detecting cytosine methylation state in DNA where primers are attached to a solid phase during amplification (see pg.9 and 11-12). Olek et al. also teach amplificates bearing labels including radionuclides, or detachable mass labels which can be detected in a mass spectrometer (see pg. 12, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to use radionuclides as labels or removable mass labels, or conduct the method with the primers bound to a solid phase of Olek et al. in the method of Herman et al. and Radlinska et al., because such techniques were alternative methods to using fluorescent labels in analyzing cytosine methylation patterns in DNA, as demonstrated by Olek et al. Since Herman et al. demonstrate the benefits of using labels to detect amplificates, as well as using solid phases in the isolation and detection of amplificates, and Olek et al. demonstrate that it was conventional in the art at the time of the invention to use removable mass labels which can be detected in a mass spectrometer, radionuclide labels, and to also conduct amplification where the primers are bound to a solid phase, it would have been *prima facie* obvious to one skilled in the

art to substitute label and detection technique for the other to achieve the predictable result of detecting cytosine methylation patterns in DNA labeled various types of labels.

Double Patenting

15. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

2. Claims 1, 11-20, 23, 25-29, 31-36 and 40 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-9, 13, 14, 17-22, 31-36 and 39 of U.S. Patent No. 7,229,759 in view of Radlinska et al. (Acta Microbiol. Polonica, vol. 47, pp. 327-334, 1998; cited in the previous office action).

A) Claim 1 of the '759 patent is drawn to a method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted: a genomic DNA sample, which comprises the DNA to be investigated as well as background DNA, is chemically treated in such a way that all unmethylated cytosine bases are converted to uracil,

while 5-methylcytosine bases remain unchanged; the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides, as well as a polymerase and at least one blocking oligonucleotide or PNA oligomer which preferentially binds to a 5'-CG-3' dinucleotide or a 5'-TG-3' dinucleotide or a 5'-CA-3' dinucleotide on the background DNA, whereby the DNA to be investigated is preferentially amplified over the background DNA as the template, and the amplified products are analyzed and the methylation status in the DNA to be investigated is concluded from the presence of an amplified product and/or from the analysis of the amplified product.

Therefore claim 1 of the '759 patent differs from the instant claims 1 and 15 by the absence of the limitation regarding the concentration of either the dCTP or dGTP nucleotides.

Claims 2-9, 13, 14, 17-22, 31-36 and 39 of the '759 patent have limitations identical or very close in scope to the instant claims 11-18, 19, 20, 23, 25-29, 31-36 and 40, respectively,

B) Claims of the '759 patent not teach reaction mixtures where the concentration of either dCTP or dGTP is at most half the concentration of the average initial concentration of the other nucleotides.

C) Radlinska et al. teach detection of 5-methylcytosines by bisulfite conversion and subsequent amplification using reaction mixtures entirely lacking dGTP (Abstract; page 325, paragraphs 3-5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the method of Radlinska et al. to detect the unmethylated cytosines in the method of the '759 patent. The motivation to do so is provided by Radlinska et al. (page 329, second paragraph):

"The main difference between our new method and the original genomic sequencing protocol relies on direct localization of 5-MeC in the primary product of the bisulfite treatment instead of the secondary product (of PCR amplification). The primer extension mix contains only three deoxynucleotides (dATP, dCI'P, dTTP) and lacks dGTP. Elongation of the synthesis product is terminated selectively when 5-MeCs occur in template DNA, since those are the only positions where cytosines were not converted to uracil by bisulfite treatment of the template strand and dGTP is needed to continue the primer extension (Fig. 1). Therefore distinct bands appearing in the ran-off lane in positions corresponding to the nucleotide complementary to the base following 5-MeC on the template strand allow direct localization of the methylated residues (Fig. 2)."

16. No claims are free of the prior art.

Conclusion

3. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Teresa E Strzelecka/
Primary Examiner, Art Unit 1637
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